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STABITY INDICATING DISSOLUTION METHOD DEVELOPMENT FOR ESTIMATION OF METHYLDOPA AND HYDROCHLOROTHIAZIDE IN COMBINE DOSAGE FORM

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Abstract:

The aim of this work was to develop validate a dissolution test for Methyldopa and Hydrochlorothiazide in combination tablets using spectrophotometric method. The dissolution established conditions were 900 mL of 0.1M HCl pH 1.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method the areas of solution were recorded at 274-284 nm and266-276 nm for Methyldopa *and Hydrochlorothiazide respectively. It can be concluded that the method developed consists in an efficient alternative for assay of dissolution for tablets. The method was validated to meet requirements for a global regulatory filing which includes linearity, precision, accuracy robustness and ruggedness. In addition, filter suitability and drug stability in medium were demonstrated*

Keywords: *In vitro release, Stability, Dissolution study of methyldopa and Hydrochlorothiazide, Spectrophotometry, Area under curve method, Validation.*

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INTRODUCTION:

Methyldopa (MD) (Fig. 1) is 3-(3, 4dihydrophenyl)-2-Methyl-L-alanine sequihydrate is Chemical name of methyldopa [1]. It is White to yellowish white, Fine powder which may contain friable lumps it is slightly soluble in water, very slightly soluble in Ethanol (95%), practically insoluble in chloroform and in ether. It is freely soluble in dilute hydrochloric acid [2].

Hydrochlorothiazide (HCTZ) (Fig .2) is 6-chloro-3, 4dihydro-2H-1, 2, 4, benzathiadiazine-7-suiphonamide [3]. It is White or almost white, crystalline powder, odorless. Soluble in acetone, sparingly soluble in ethanol (95%).Very slightly soluble in water, it dissolves in dilute solution of alkali hydroxides [4]. Literature survey revealed UV-Visible spectrophotometric methods such as simultaneous equation method, Dual Wavelength method [5,6] and RP-HPLC [7,8] for the estimation of MD and HCTZ alone or in combination with other drugs. No method has been reported for this combination by using this mobile phase. The present work therefore emphasizes on the quantitative estimation of MD and HCTZ in bulk and pharmaceutical formulation by HPLC. The proposed method was validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines [9,10].

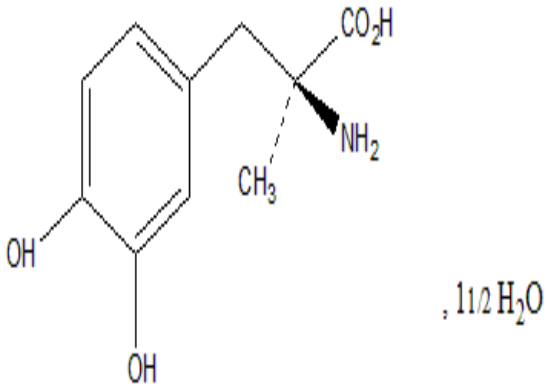

Fig. 1: Chemical Structure of Methyldopa

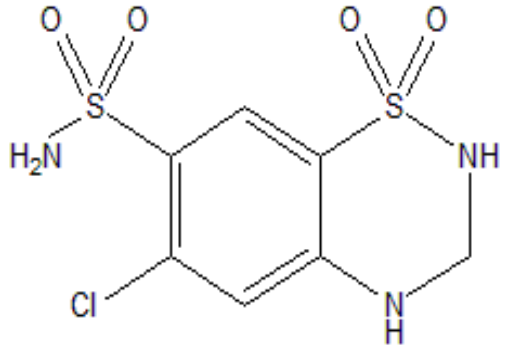

Fig. 2: Chemical Structure of Hydrochlorothiazide

MATERIAL AND METHODS:

Instrumentation

Dissolution test was performed in a ELECTROLAB (VK7025) Model (TDT-06L) [11] dissolution apparatus, multi-bath (n=6), in accordance to USP Pharmacopoeia general method. The medium were vacuum degassed under in house vacuum and weremaintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Visiblespectrophotometer (Model: UV 1800,Shimadzu] with a fixed slit width (2 nm)using 1.0 cm quartz cell was used for all absorbance measurements. Elico pH analyzer (Model: Elico 11610) was used to determine the pH of all solutions.

Chemicals

Pharmaceutically pure sample of Methyldopa and Hydrochlorothiazide obtain form Flamigo Private Ltd.Nanded & Ajanta pharma. Chitegaon. Formulations of Methyldopa and Hydrochlorothiazide Aldoril tablet(250mg of MD+25mg of HCTZ) purchased from local market.

Method for stability indicating dissolution media selection and for dissolution study

Stability studies

In stability study nine dissolution media were selected and prepared such as distilled water, 0.1M HCl, Acetate buffer 5.5, and 6.8 phosphate buffers as per USP guidelines [United States Pharmacopoeia XXX, 2007]. Stock solutions of MD and HCTZ were prepared by dissolving accurately weighed 10 mg of both drug in 100 ml of distilled water, 0.1M HCl, Acetate buffe 5.5, and 6.8 phosphate buffers separately to obtain 100 µg/ml solutions. All the solutions were sonicated using ultrasonicater to dissolve the drug. From these solutions 1 ml was pipette out into 10 ml volumetric flask and diluted with the same solvent system up to the mark to obtain 10 µg/ml solutions. Two sets of 10 µg/ml solutions of MD and HCTZ are prepared and stability was tested in the above prepared dissolution media at room temperature (RT) and 37ºC in an incubator (Thermo lab) for 48 hrs separately. These samples are studied at 0, 24 and 48 hrs interval by using a double-beam UV-visible spectrophotometer (shimadzu UV1800) connected to UV probe software. The λmax and absorbance value was measured for all the solutions and deviations in the values are recorded which indicates stability in 0.1M HCL. These stable dissolution Medias are used for further dissolution studies of both the drugs.

Table No 1: Media Selection of MD

Medium	0 HOUR		24 HOUR		48 HOUR		% CV
	λmax	Absorbance	λmax	Absorbance	λmax	Absorbance	
Distilled water	279.40	0.145	279.40	0.140	279.40	0.137	2.87307
0.1M HCL	279.60	0.150	279.60	0.155	279.85	0.158	4.760393
Buffer (6.8)	280	0.121	280	0.126	280	0.144	9.281
Acetate Buffer (5.5)	279.90	0.131	280	0.135	280	0.145	5.263

Table No 2: Media Selection of HCTZ

Medium	0 HOUR		24 HOUR		48 HOUR		% CV
	λmax	Absorbance	λmax	Absorbance	λmax	Absorbance	
Distilled water	271.40	0.698	271.20	0.742	271.30	0.791	6.2553
0.1M HCL	271.40	0.580	271.20	0.583	271.20	0.585	5.9222
Buffer (6.8)	271.40	0.687	271	0.700	271	0.676	1.74705
Acetate Buffer (5.5)	271.20	0.523	271	0.566	271	0.591	6.15192

Simultaneous Spectrophotometric Determination of Methydopa and Hydrochlorothiazide by Area under Curve Method

The release of kinetic of Methyldopa and Hydrochlorothiazide from tablets was studied by conducting dissolution tests. Dissolution tests performed using USP type 2 dissolution apparatus and 900ml of 0.1N Hcl at $37^{\pm}$ 0.5^{0}c at 50rpm 10ml sample were withdrawn at the intervals of 5,10,,15,20,25,30,35,40,45,60min. Sampling was carried out and every time replaced with fresh 10ml with 0.1N Hcl. The areas of solution were recorded at 274-284 nm and266-276 nm for MD and HCTZ respectively using 0.1N Hcl as blank. The dissolution studies were performed in triplicate (n=3).

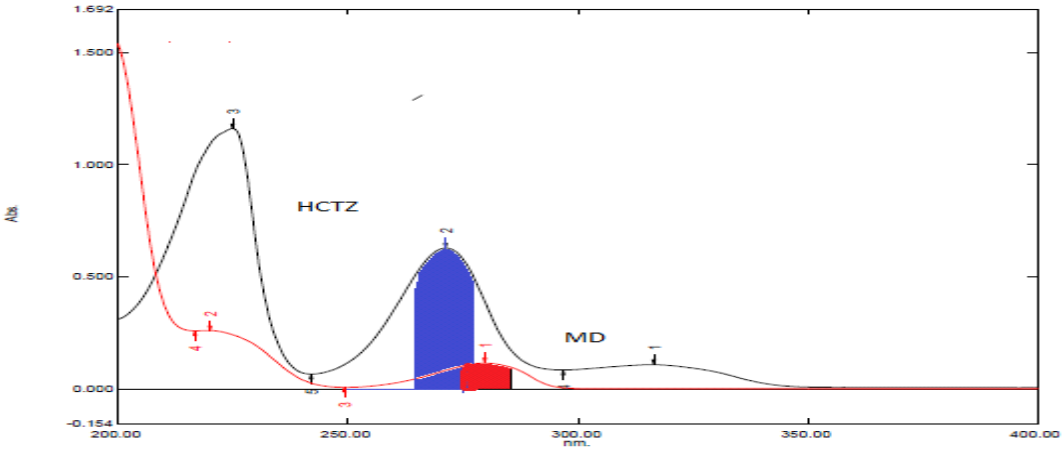

Fig 3: Overlain Spectra of MD and HCTZ

Table 3: Calculation by AUC Method

Sr. No	Sampling Time	Area at		Percentage Released (%)	
		274-284	266-276		
1		MD	HCTZ	MD	HCTZ
2	5	0.128	0.089	48.4	47.21
3	10	0.148	0.108	54.63	56.58
4	15	0.175	0.138	61.82	64.99
5	20	0.224	0.164	68.85	71.65
6	25	0.235	0.177	79.8	77.41
7	30	0.241	0.189	85.59	83.58
8	35	0.252	0.211	90.47	92.7
9	40	0.259	0.221	99.90	99.79
10	45	0.63	0.225	100.2	100.4
11	60	0.255	0.218	95.48	96.59

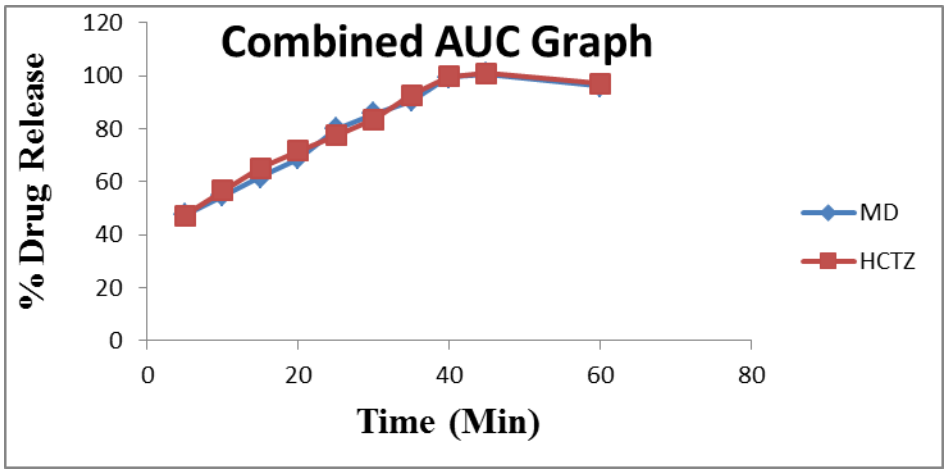

Fig.4: AUC Graph

Method Validation

Linearity

The linearity of Methyldopa response was evaluated from the range of 10-60µg/ml. And that for Hydrochlorothiazide was 2-14µg/ml and showed a good correlation coefficient. To assess linearity, the standard curves Methyldopa and Hydrochlorothiazide are constructed by plotting concentration (µg/ml) verses absorbance .

Precision

The precision of the method is evaluated by measuring the repeatability in two different UV Vis spectrophotometers

Recovery

The accuracy is evaluated by applying proposed method to the analysis of mixture of the tablet and with known amount of the Methyldopa and Hydrochlorothiazide working standard. Corresponding to the concentration of 80, 100,and 120% which were subjected to dissolution test conditions described above

Ruggedness

Ruggedness of the method is determined by carrying out the analysis by two different analysis and the respective dissolution values are calculated

Stability indicating assay method

Preparation of stock solution

Standard stock solution of Methyldopa & Hydrochlorothiazide was prepared by dissolving 10mg of Methyldopa & Hydrochlorothiazide in 100ml of 0.1N Hcl which gives 100µg/ml solution.

Preparation of working solution

From the above stock solution 1ml was transferred into 10ml volumetric flask &The volume made was up to mark with 0.1N Hcl to give 10µg/ml.

Preparation of Blank solution

\In separate 10ml volumetric flask, each containing 5ml of solvents used for dedradation such as 0.1N Hcl, 1N Hcl, 0.1N NaoH, 1N NaoH& 3% H_2O_2&Neutrlise with solvent & Volume was made up with 0.1N Hcl.

Acid degradation

10 ml volume flask containing 3 ml stock solution of Methyldopa & Hydrochlorothiazide 5 ml (0.1 & 1 N Hcl) , was added & heated at 60^0c for 3 hours.Which was then neutralized with proper solvent and final volume made up to mark with NaoH to form solution 10µg/ml of drug stock solution.

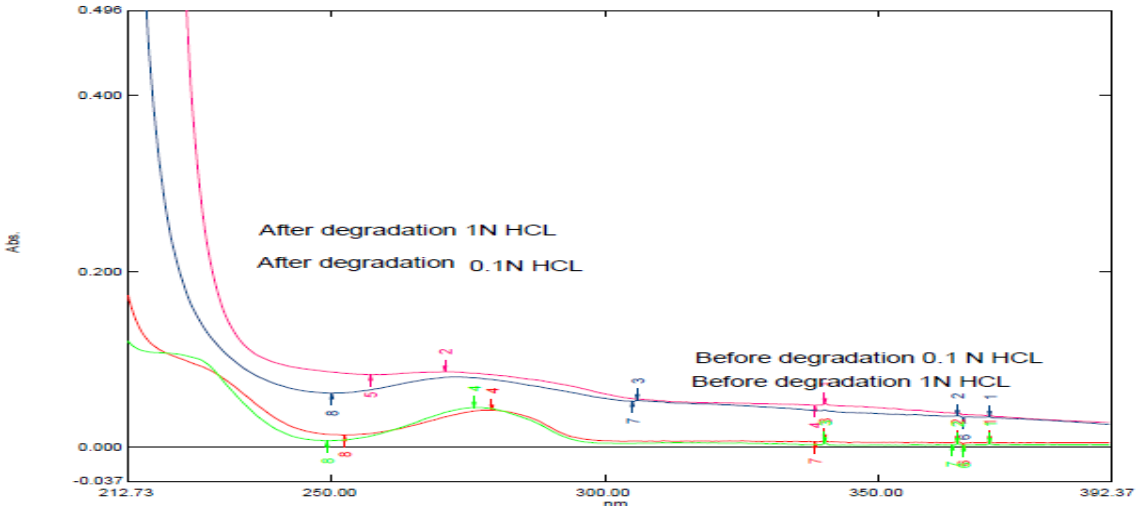

Fig 5: Acid degradation of MD 10µg/ml

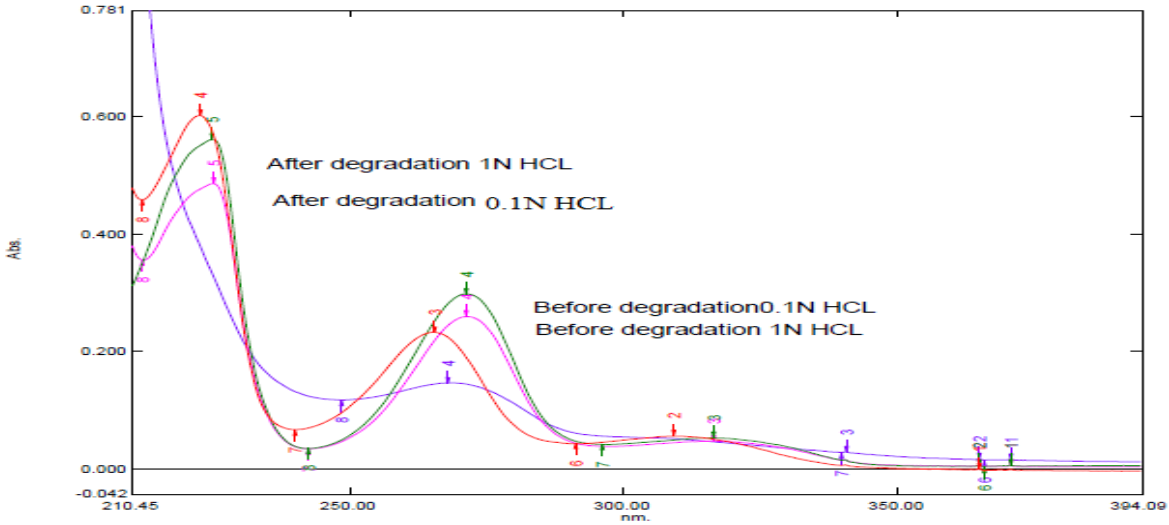

Fig 6: Acid degradation HCTZ 10µg/ml

Alkali degradation

10 ml volumetric flack containing 3 ml stock solution of Methyldopa & Hydrochlorothiazide, 5 ml (0.1 & 1N NaOH) was added & heated at 60^0c for 3 hours.Which was then neutralized with proper solvent and final volume made up to mark with 0.1 N Hcl to form solution 10µg/ml of drug stock solution.

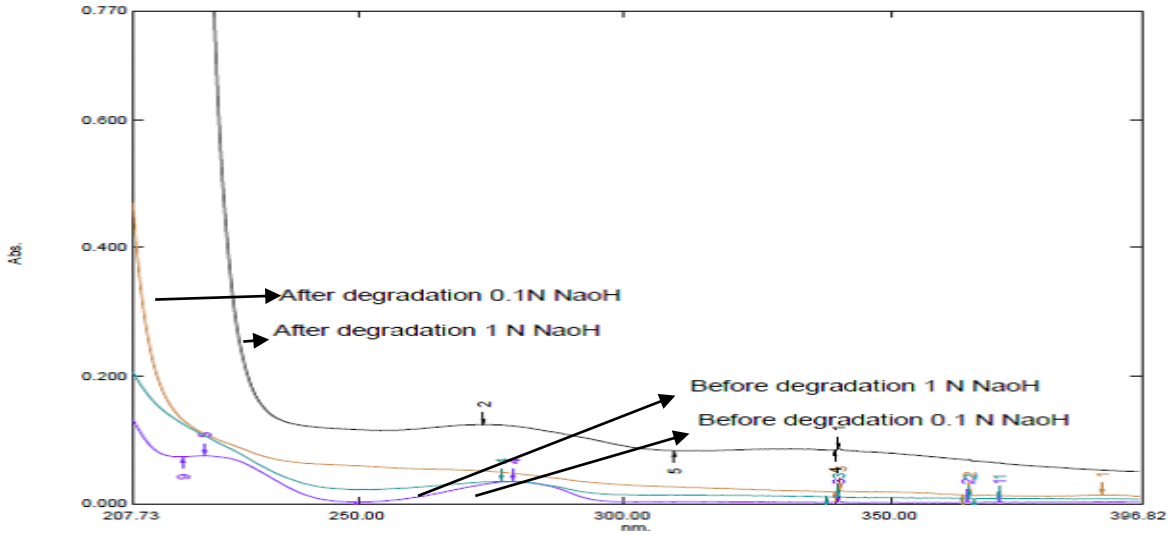

Fig 7: Alkali degradation of MD 10µg/ml

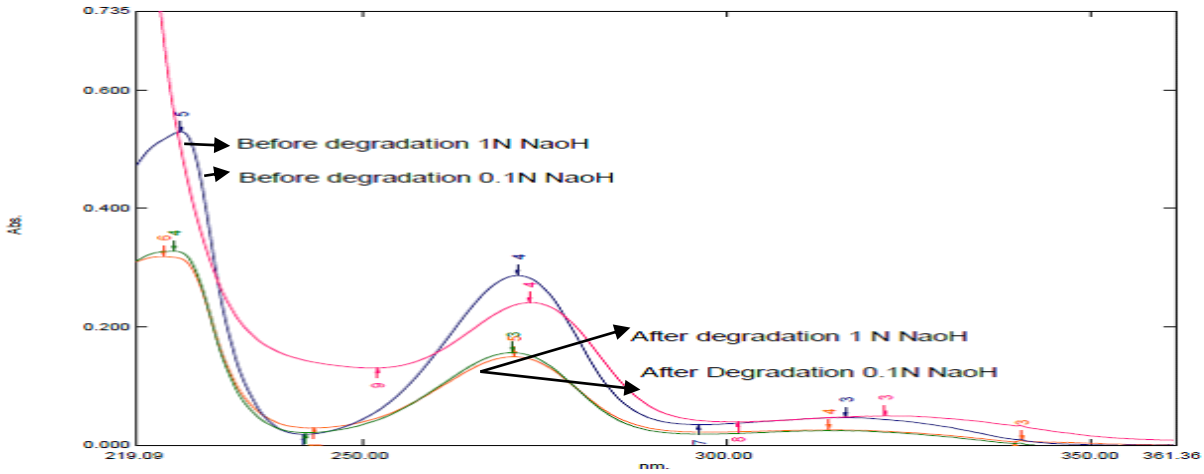

Fig 8: Alkali degradation of HCTZ10µg/ml

Oxidation degradation

10 ml volumetric flack containing 3 ml stock solution of Methyldopa & Hydrochlorothiazide, 5 ml 3% H_2O_2 was added & Kept in 3hr for room temperature and final volume made up to mark with NaOH to form solution 10µg/ml of drug stock solution.

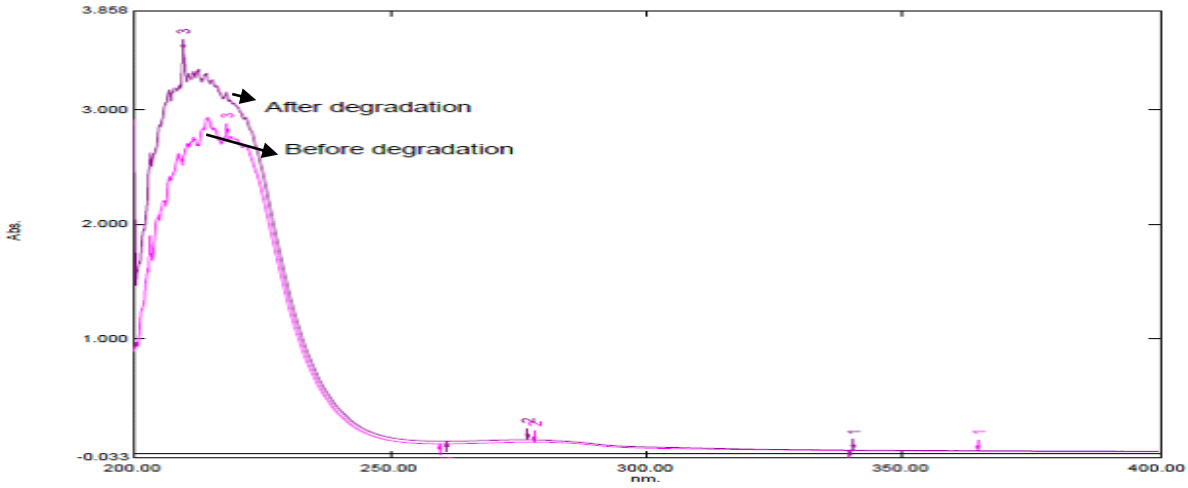

Fig 9 : Oxidationdegradation MD 10µg/ml

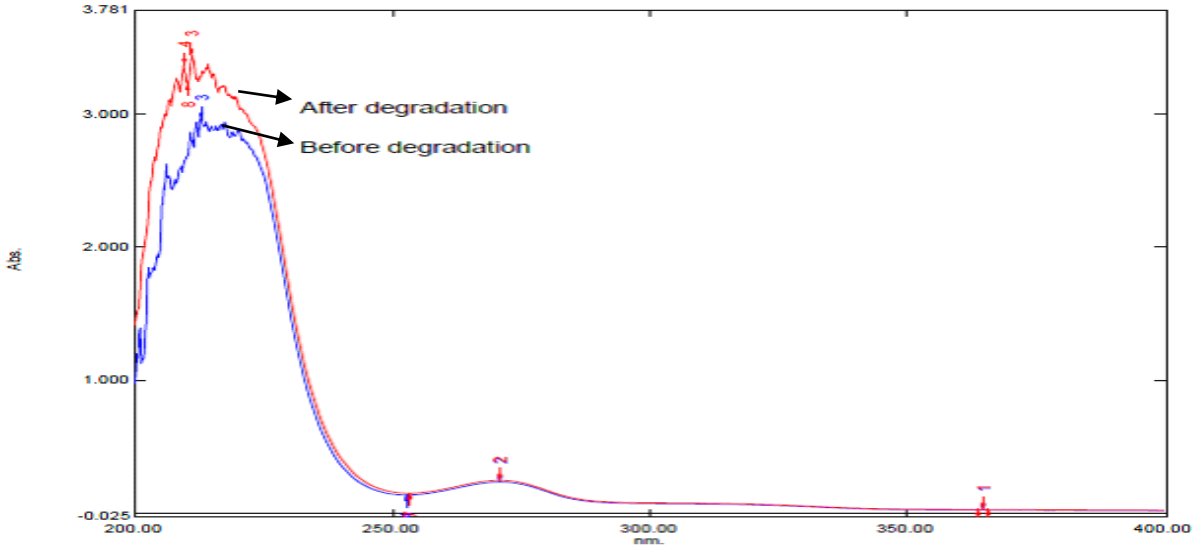

Fig 10: Oxidative degradation HCTZ 10µg/ml

Thermal degradation

50mg of MD & HCTZ was weighted & kept in the oven & temperature was maintained at 80^0c for 3hrs from this 1 mg of exposed MD & HCTZ was transferred in 100ml volumetric flack and final volume made upto 0.1N Hcl.

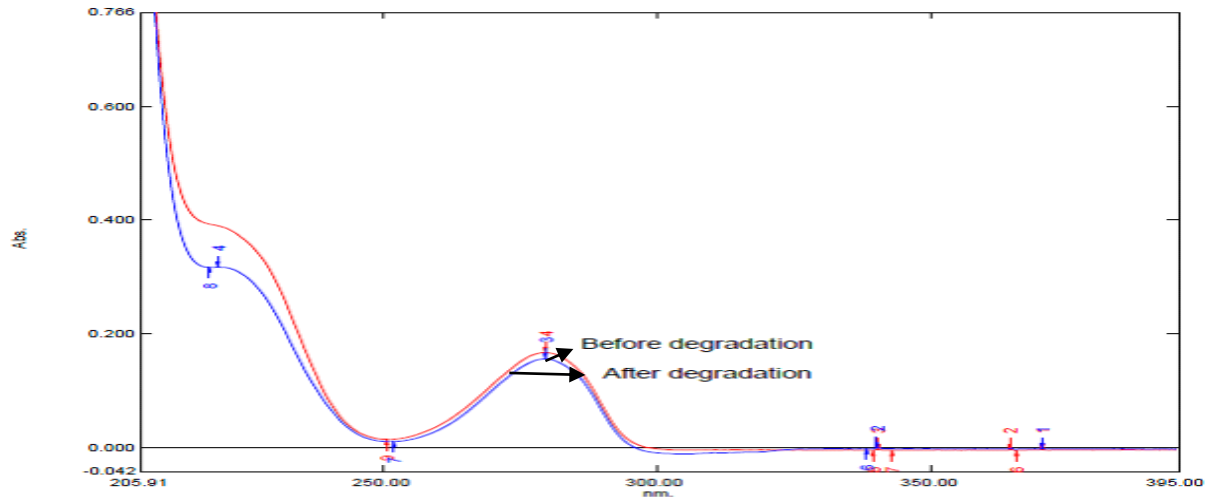

Fig 11: Thermal degradation MD10µg/ml

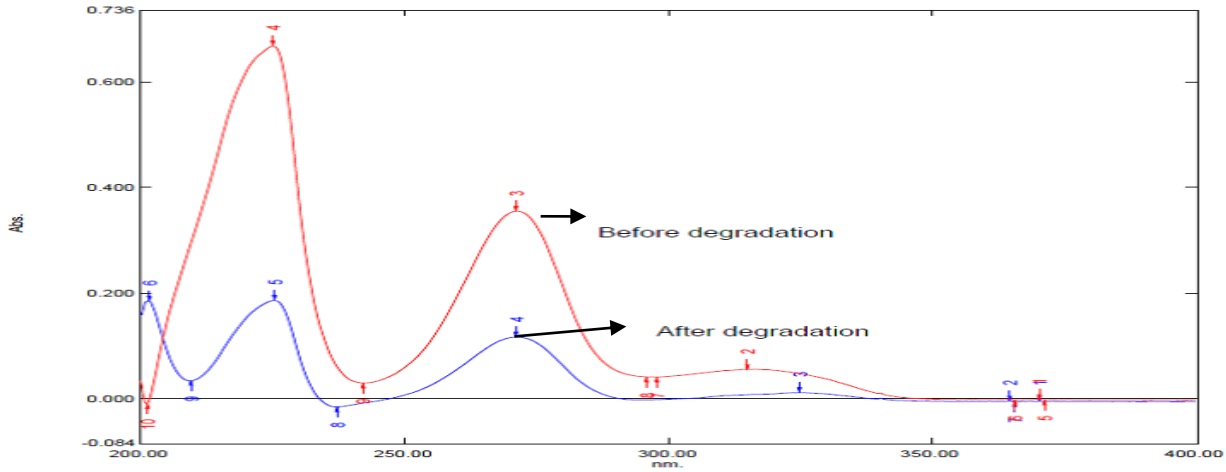

Fig 12: Thermal degradation HCTZ 10µg/ml

Photolytic Degradation

50mg of MD & HCTZ was exposed in sunlight & degradation drug not achieved. From this 1mg exposed MD & HCTZ was transfereed in 100ml volumetric flack and final volume made with 0.1N Hcl.

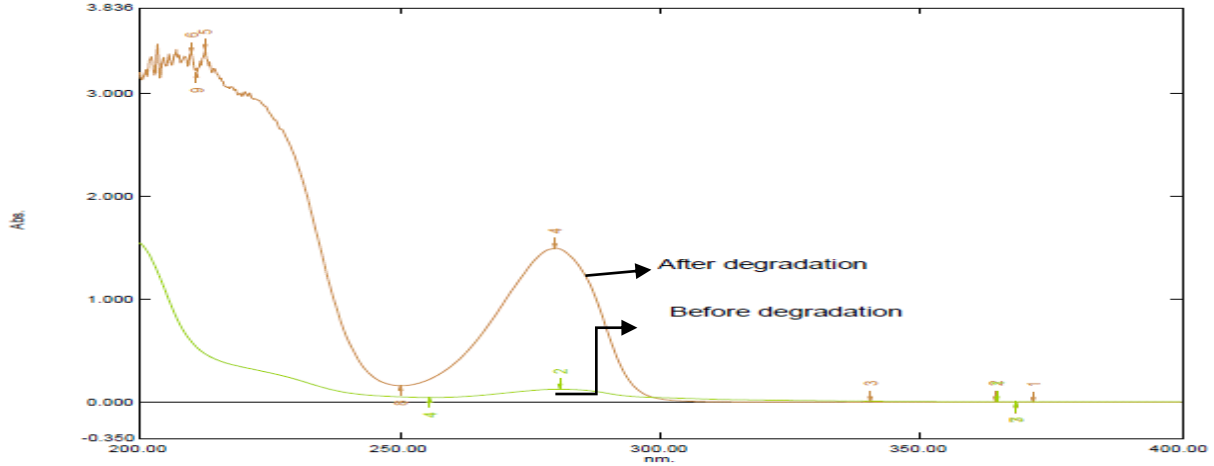

Fig 13: Photolytic degradation MD10µg/ml

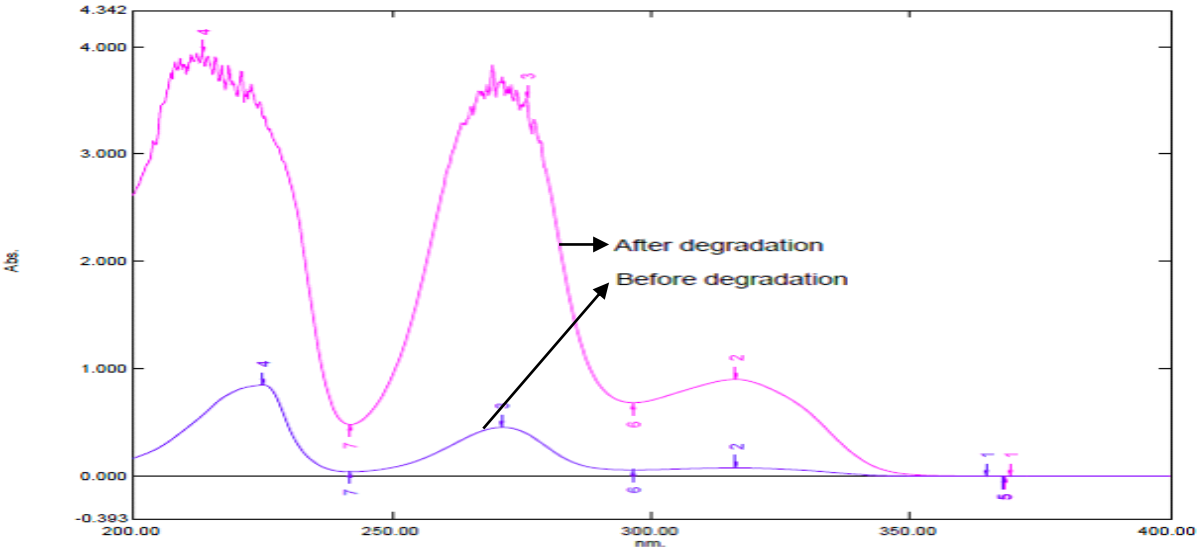

Fig14: Photolytic degradation HCTZ10µg/ml

Table 4: Repeatability and intermediate precision of the dissolution method

Method	Mean		Standard deviation		Coefficient of variation		Standard error	
Intraday								
AUC	**MD**	**HCTZ**	**MD**	**HCTZ**	**MD**	**HCTZ**	**MD**	**HCTZ**
	99.94	**99.94**	**0.036**	**0.035**	**0.036**	**0.0351**	**0.0208**	**0.0202**
Interday								
	99.93	**99.96**	**0.0404**	**0.0152**	**0.0404**	**0.015**	**0.0233**	**0.0088**

	Level of % Recovery	Amt. Present (mcg/tab)		Amt.of standard added(mcg/ta)		Total Amt. Recoverd (mcg)		% Recovery	
		MD	HCTZ	MD	HCTZ	MD	HCTZ	MD	HCTZ
AUC	80	27	2.7	21.6	2.16	48.6	4.86	99.78	99.16
	100	27	2.7	27	2.7	54	5.4	99.59	100.13
	120	27	2.7	32.4	3.24	59.4	5.94	99.83	99.81

Method	Analyst1		Analyst2	
	MD	**HCTZ**	**MD**	**HCTZ**
AUC	99.81	99.84	99.70	99.78
Mean	99.72	99.78	99.79	99.85

n=3 n=3, SD= standard deviation %RSD= Relative standard deviation SE= Standard error

CONCLUSION:

The Area under curve Method requires only measurement of area at selected wavelength. Area under curve, have been developed for determination of MD & HCTZ in tablet dosage form. From the statistical result, it can be concluded that this method was accurate, precise, robust and reproducible. A simple dissolution test developed and validated for Methyldopa and Hydrochlorothiazide tablets are considered satisfactory. The conditions that allowed the dissolution determination ware 900 mL of 0.1 M HCl at 37.0 ± 0.5 ºC, paddle apparatus, 50 rpm stirring speed and filtration with 0.45 µ cellulose acetate membrane filters. In these conditions, Methyldopa and Hydrochlorothiazide stability is good. The percent drug delivery is higher than 90% in 40 minutes for both drugs in evaluated products. Therefore, the proposed method was successfully applied and suggested for the quality control

studies of Methyldopa and Hydrochlorothiazide pharmaceutical dosage forms contributing to assure the therapeutic efficacy of the drug.

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